

# **UCLA**

## **UCLA Previously Published Works**

### **Title**

Genome-wide association study identifies eight loci associated with blood pressure.

### **Permalink**

<https://escholarship.org/uc/item/4xk0g9nn>

### **Journal**

Nature genetics, 41(6)

### **ISSN**

1061-4036

### **Authors**

Newton-Cheh, Christopher  
Johnson, Toby  
Gateva, Vesela  
et al.

### **Publication Date**

2009-06-01

### **DOI**

10.1038/ng.361

Peer reviewed



Published in final edited form as:

Nat Genet. 2009 June ; 41(6): 666–676. doi:10.1038/ng.361.

## Eight blood pressure loci identified by genome-wide association study of 34,433 people of European ancestry

A full list of authors and affiliations appears at the end of the article.

### Abstract

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use:[http://www.nature.com/authors/editorial\\_policies/license.html#terms](http://www.nature.com/authors/editorial_policies/license.html#terms)

Correspondence to: Gonçalo Abecasis, Center for Statistical Genetics, Department of Biostatistics, University of Michigan School of Public Health, 1420 Washington Heights, Ann Arbor, MI 48109, Phone 734 763 4901, Fax 734 615 8322, Email: goncalo@umich.edu, Mark Caulfield, Clinical Pharmacology, William Harvey Research Institute, Barts and The London, Charterhouse Square, London, EC1M 6BQ, Tel: 02078823402, Fax: 02078823408, Email: m.j.caulfield@qmul.ac.uk, Patricia Munroe, Clinical Pharmacology, William Harvey Research Institute, Barts and The London, Charterhouse Square, London, EC1M 6BQ, Tel: 02078823410, Fax: 02078823408, email: p.b.munroe@qmul.ac.uk, Christopher Newton-Cheh, Center for Genetic Research, Cardiovascular Research Center, Massachusetts General Hospital, 185 Cambridge Street, CPZN 5.242, Boston, MA 02114, Tel: +1 617 643 3615, Fax: 617 249 0127, Email: cnewtoncheh@chgr.mgh.harvard.edu.

<sup>94</sup>These authors contributed equally.

<sup>95</sup>These authors contributed equally.

Author contributions (alphabetical)

**Project conception, design, management:** ARYA: M.L.B., C.S.U.; BLSA: L.F.; B58C-T1DGC: D.P.S.; B58C-WTCCC: D.P.S.; BRIGHT: M.Brown, M.C., J.M.C., A. Dominiczak, M.F., P.B.M., N.J.S., J.W.; CoLaus: J.S.B., S.Bergmann, M.Bochud, V.M. (PI), P.Vollenweider (PI), G.W., D.M.W.; DGI: D.A., C.N.-C., L.G.; EPIC-Norfolk-GWAS: I.B., P.D., R.J.F.L., M.S.S., N.J.W., J.H.Z. EPIC-Italy: S. Polidoro, P.Vineis. Fenland Study: R.J.F.L., N.G.F., N.J.W.; Finrisk97: L.P., V.S.; FUSION: R.N.B., M.Boehnke, F.S.C., K.L.M., L.J.S., T.V., J.T.; InCHIANTI: S. Bandinelli, L.F.; KORA: A. Döring, C.G., T.I., M.L., T.M., E.O., H.E.W. (PI); LOLIPOPOP: J.C.C., P.E., J.S.K. (PI); MDC-CC: G.B., O.M.; MPP: G.B., O.M.; MIGen: D.A., R.E., S.K., J.M., O.M., C.J.O., V.S., S.M.S., D.S.S.; METSIM: J.K., M.L.; NFBC1966: P.E., M.-R.J.; PREVEND: P.E.d.J. (PI), G.N., W.H.v.G.; PROCARDIS: R.C., M.F., A.H., J.F.P., U.S., G.T., H.W. (PI); PROSPECT-EPIC: N.C.O.-M., Y.T.v.d.S.; SardiNIA: E.G.L., D.S.; SHIP: M.D., S.B.F., G.H., R.L., T.R., R.R., U.V., H.V.; SUVIMAX: P.M.; TwinsUK: P.D., T.D.S. (PI); UHP: D.E.G., M.E.N.

**Phenotype collection, data management:** ARYA: M.L.B., C.S.U.; B58C-T1DGC: D.H., W.L.M., D.P.S.; B58C-WTCCC: D.H., K.P., D.P.S.; BRIGHT: M. Brown, M.C., J.M.C., A.Dominiczak, M.F., P.B.M., N.J.S., J.W.; CoLaus: G.W.; DGI: L.G., O.M.; EPIC-Italy: P. Vineis (PI); Finrisk97: P.J., M.P., V.S.; FUSION: J.T., T.V.; KORA: A. Döring, C.G., T.I.; MDC-CC: O.M.; MPP: O.M., P.N.; MIGen: D.A., R.E., S.K., J.M., O.M., C.J.O., S.M.S., D.S.S., V.S.; NFBC1966: A.-L.H., M.-R.J., A.P.; PREVEND: P.E.d.J., G.N., P.v.d.H., W.H.v.G.; PROCARDIS: R.C., A.H., U.S., G.T.; PROSPECT-EPIC: N.C.O.-M., Y.T.v.d.S.; SardiNIA: S.S.N., A.S.; SHIP: M.D., R.L., R.R., H.V.; SUVIMAX: P.G., S.H.; TwinsUK: F.M.W.; UHP: D.E.G., M.E.N.

**Genome-wide, validation genotyping:** B58C-T1DGC: W.L.M.; B58C-WTCCC: W.L.M.; DGI: D.A., O.M., M.O.-M.; EPIC-Norfolk-GWAS: I.B., P.D., N.J.W., J.H.Z.; EPIC-Norfolk-replication: S.A.B., K.-T.K., R.J.F.L., R.N.L., N.J.W.; EPIC-Italy: G.M.; EPIC-Italy: A.A., A.d.G., S.G., V.R.; Finrisk97: G.J.C., C.N.-C.; FUSION: L.L.B., M.A.M.; KORA: T.I., T.M., E.O., A.P.; MDC-CC: O.M., M.O.-M.; MPP: O.M., M.O.-M.; NFBC1966: P.E., N.B.F., M.-R.J., M.I.M., L.P.; PREVEND: G.N., P.v.d.H.; W.H.v.G.; PROCARDIS: S.C.H., G.M.L., A.-C.S.; SardiNIA: M.U.; SHIP: F.E., G.H., A.T., U.V.; SUVIMAX: I.G.G., S.C.H., G.M.L., D.Z.; TwinsUK: P.D.

**Data analysis:** BLSA: T.T.; B58C-T1DGC: D.H., S.H., D.P.S.; B58C-WTCCC: P.R.B., D.H., K.P., D.P.S., M.D.T.; B58C-T1DGC: D.H., S.H., D.P.S.; BRIGHT: S.J.N., C.W., E.Z.; CoLaus: S. Bergmann, M. Bochud, T.J., N.L., K.S., X.Y., DGI: O.M., C.N.-C., M.O.-M., B.F.V.; EPIC-Norfolk-GWAS: R.J.F.L., J.H.Z.; EPIC-Norfolk-replication: S.A.B., K.-T.K., R.J.F.L., R.N.L., N.J.W.; EPIC-Italy: S.G., G.M., S. Panico, S. Polidoro, F.R., C.S., P. Vineis; Fenland Study: J.L.; Finrisk97: C.N.-C.; FUSION: A.U.J., L.J.S., H.M.S., C.J.W.; InCHIANTI: T.T.; KORA: S.E., C.G., M.L., E.O.; LOLIPOPOP: J.C.C.; MDC-CC: O.M., M.O.-M.; MPP: O.M., M.O.-M.; MIGen: R.E., G.L., I.S., B.F.V.; NFBC1966: L.C., P.F.O.; PREVEND: H.S., P.v.d.H.; PROCARDIS: M.F., A.G., J.F.P.; SardiNIA: V.G., S.S., P.S.; SHIP: F.E., G.H., A.T., U.V.; SUVIMAX: S.C.H., T.J., P.M.; TwinsUK: N.S., F.Z., G.Z.

**Analysis group:** G.R.A., M.C., V.G., T.J., P.B.M., C.N.-C., M.D.T., L.V.W.

**Writing group:** G.R.A., M.C., P.E., V.G., T.J., P.B.M., C.N.-C., M.D.T.

### COMPETING INTERESTS STATEMENT

The following authors declare the following potential conflicts of interest: N.L., V.M., K.S., D.M.W. and X.Y. are all full-time employees at GlaxoSmithKline. P. Vollenweider and G.W. received financial support from GlaxoSmithKline to assemble the CoLaus study. No other authors reported conflicts of interest.

Elevated blood pressure is a common, heritable cause of cardiovascular disease worldwide. To date, identification of common genetic variants influencing blood pressure has proven challenging. We tested 2.5m genotyped and imputed SNPs for association with systolic and diastolic blood pressure in 34,433 subjects of European ancestry from the Global BPgen consortium and followed up findings with direct genotyping (N = 71,225 European ancestry, N=12,889 Indian Asian ancestry) and *in silico* comparison (CHARGE consortium, N=29,136). We identified association between systolic or diastolic blood pressure and common variants in 8 regions near the *CYP17A1* ( $P=7\times 10^{-24}$ ), *CYP1A2* ( $P=1\times 10^{-23}$ ), *FGF5* ( $P=1\times 10^{-21}$ ), *SH2B3* ( $P=3\times 10^{-18}$ ), *MTHFR* ( $P=2\times 10^{-13}$ ), *c10orf107* ( $P=1\times 10^{-9}$ ), *ZNF652* ( $P=5\times 10^{-9}$ ) and *PLCD3* ( $P=1\times 10^{-8}$ ) genes. All variants associated with continuous blood pressure were associated with dichotomous hypertension. These associations between common variants and blood pressure and hypertension offer mechanistic insights into the regulation of blood pressure and may point to novel targets for interventions to prevent cardiovascular disease.

The World Health Organization estimated that, in 2005, the annual death toll from cardiovascular disease reached 17.5 million worldwide<sup>1–3</sup>. Increases in systolic and diastolic blood pressure (SBP, DBP), even within the normal range, have a continuous and graded impact on cardiovascular disease risk and are major contributors in half of all cardiovascular deaths<sup>2,3</sup>. Lifestyle influences, including dietary sodium intake, alcohol excess, elevated body mass index and lack of exercise, are known to increase blood pressure<sup>4</sup>. Studies of familial aggregation suggest that there is also a substantial heritable component to blood pressure<sup>5</sup>. Studies of rare Mendelian disorders of hypertension and hypotension have produced the most significant progress toward understanding the heritable basis of blood pressure, showing that mutations in genes influencing renal salt handling can have a severe impact on blood pressure<sup>6</sup>. Detailed study of these genes has identified rare variants (minor allele frequency [MAF] <0.1%) that impact blood pressure in the general population<sup>7</sup> and evolving evidence suggests a potential role for common variation in some of the same genes<sup>8–10</sup>.

Identification of common variants affecting blood pressure using genome-wide association studies (GWAS) has proved challenging, compared to other common complex disorders<sup>11,12</sup>. However, meta-analysis of multiple studies with large total sample sizes has the potential to facilitate detection of variants with modest effects. We therefore formed the Global Blood Pressure Genetics (Global BPgen) consortium and conducted meta-analysis of GWAS in 34,433 individuals of European ancestry with SBP and DBP measurements (stage 1), followed by large-scale direct genotyping (stage 2a) and *in silico* (stage 2b) analyses (Supplementary Figure 1). Our analyses identified eight loci demonstrating genome-wide significant association with systolic or diastolic blood pressure, with each locus also providing substantial evidence for association with hypertension.

## RESULTS

### Genome-wide association for blood pressure

Global BPgen includes 17 cohorts of European ancestry ascertained through population-based sampling or case-control studies. In our primary analysis (stage 1), we examined

individuals aged 70 years from 13 population-based studies and from control groups from 4 case-control studies (Table 1). Individuals treated for hypertension were imputed to have 15 mm Hg higher SBP and 10 mm Hg higher DBP than the observed measurements as this has been shown to reduce bias and improve statistical power<sup>13</sup>. SBP and (separately) DBP measures were each adjusted for age, age<sup>2</sup>, body mass index and any study-specific geographic covariates within cohort- and gender-specific regression analyses. Genome-wide SNP genotyping was performed on a variety of platforms and subjected to standard quality control measures (Methods, Supplementary Table 1). Genotypes for ~2.5M autosomal SNPs in the HapMap CEU sample were then imputed in each study and tested for association under an additive genetic model with SBP and DBP separately. Test statistics from association analysis of SBP and DBP from each cohort were adjusted using genomic control<sup>14</sup> to avoid inflation of results due to inter-individual relatedness or residual population stratification, and to ensure good calibration of test statistics. Meta-analysis of results was performed using inverse variance weights. Test statistic inflation post-meta-analysis was modest ( $\lambda_{GC} = 1.08$  SBP;  $\lambda_{GC} = 1.07$  DBP); genomic control correction was applied again. The plots of test statistics against expectations under the null suggest an excess of extreme values (cohort-specific and meta-analysis quantile-quantile plots are presented in Supplementary Figure 2).

On meta-analysis of results from 34,433 individuals in stage 1, we observed 11 independent signals with  $P < 10^{-5}$  for SBP and 15 for DBP, with two results attaining  $P < 5 \times 10^{-8}$ , corresponding to genome-wide significance when adjusting for ~1m independent common variant tests estimated for samples of European ancestry (Supplementary Figure 3)<sup>15</sup>.

### Follow-up of strongest SBP and DBP signals in additional samples

To strengthen support for association we undertook two analyses. First, we selected 12 SNPs for follow-up genotyping in up to 71,225 individuals drawn from 13 cohorts of European ancestry and up to 12,889 individuals of Indian Asian ancestry from one cohort (stage 2a, Table 1, Supplementary Figure 1, Supplementary Table 2). Second, we performed a reciprocal exchange of association results for 10 independent signals each for SBP and DBP (stage 2b, Supplementary Figure 1, Supplementary Table 3) with colleagues from the Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) blood pressure consortium who had recently meta-analyzed GWAS data for SBP and DBP in 29,136 individuals, independent of Global BPgen (Table 1). Meta-analysis of the stage 1 Global BPgen GWAS and stage 2a direct and stage 2b *in-silico* association results identified genome-wide significant ( $P < 5 \times 10^{-8}$ ) associations at eight loci: 1p36 in *MTHFR*, 10q24 near *CYP17A1* and 17q21 in *PLCD3* with SBP, 4q21 near *FGF5*, 10q21 in *C10orf107*, 12q24 near *SH2B3*, 15q24 near *CYP1A2*, and 17q21 near *ZNF652* with DBP (Table 2, Figure 1, Supplementary Table 2, Supplementary Table 3, Supplementary Figure 3). Three of these loci overlap with genome-wide significant loci identified in the CHARGE analyses (10q24 for SBP and 12q24 and 15q24 for DBP).

For SBP, the strongest evidence for association was at 10q24 (rs11191548, MAF = 0.09, 1.16 mm Hg higher per major allele,  $P = 7 \times 10^{-24}$ , Table 2, Figure 1b). This SNP is part of a large cluster of associated SNPs spanning a ~430Kb region at 10q24 showing association in

our GWAS meta-analysis. The locus includes six genes, most notably *CYP17A1*, which encodes the cytochrome P450 enzyme CYP17A1 (also known as *P450c17*) that mediates steroid 17 $\alpha$ -hydroxylase and 17,20-lyase activity. The first enzymatic action is a key step in the biosynthesis of mineralocorticoids and glucocorticoids that affect sodium handling in the kidney and the second is involved in sex-steroid biosynthesis. Missense mutations in *CYP17A1* cause one form of adrenal hyperplasia characterized by hypertension, hypokalemia, and reduced plasma renin and aldosterone levels<sup>16,17</sup>. None of the five other genes/transcripts in the region (Figure 1b) is an obvious candidate for blood pressure regulation.

The second locus associated with SBP was at 1p36 (rs17367504, MAF 0.14, 0.85 mm Hg lower SBP/minor allele,  $P = 2 \times 10^{-13}$ , Table 2, Figure 1a). This SNP is located in an intron of the *MTHFR* (methylenetetrahydrofolate reductase) gene in a region with many plausible candidate genes, including: *MTHFR*, *CLCN6*, *NPPA*, *NPPB*, and *AGTRAP*. The strongest signal in the locus is 6.4kb away from and uncorrelated with rs1801133 (C677T, A222V  $r^2$  CEU = 0.06), a coding variant that has been related to higher plasma homocysteine concentration<sup>18</sup>, pre-eclampsia<sup>19</sup>, and variably hypertension<sup>20</sup>. In Global BPgen rs1801133 was associated with 0.08 mm Hg higher SBP/T allele ( $P = 0.56$ ), 0.24 mm Hg higher DBP ( $P = 0.01$ ) and an odds ratio for hypertension of 1.00 (95% CI 0.94-1.05,  $P = 0.90$ ).

The natriuretic peptides encoded by *NPPA* and *NPPB*, also located within the 1p36 associated interval, have vasodilatory and natriuretic properties and the *NPPA* knockout mouse has salt-sensitive hypertension<sup>21</sup>. A recent study found that the minor allele of rs5068 (43 kb from rs17367504,  $r^2$  CEU = 0.26), in the 3' untranslated region of *NPPA*, is associated with higher plasma atrial natriuretic peptide and B-type natriuretic peptide, as well as lower SBP, DBP and odds of hypertension<sup>22</sup>. In the Global BPgen stage 1 meta-analysis we confirmed association of the minor allele of rs5068 with 0.97 mm Hg lower SBP ( $P = 3 \times 10^{-4}$ ), 0.60 mm Hg lower DBP ( $P = 1 \times 10^{-3}$ ) and 10% lower odds of hypertension ( $P = 0.04$ ). Whether the associations of rs5068 and rs17367504 reflect the same or different underlying signals remains to be established. The less well-characterized gene *CLCN6*, also at the 1p36 locus, encodes a neuronally-expressed chloride channel that has not previously been implicated in blood pressure physiology, although rare mutations in other renally-expressed chloride channels have been associated with extremes of blood pressure<sup>23,24</sup>. Lastly, *AGTRAP* (encoding angiotensin II receptor-associated protein) negatively regulates angiotensin II signaling by interacting with the angiotensin II type 1 receptor, a critical component of the renin-angiotensin-aldosterone system and a target of antihypertensive therapy<sup>25</sup>.

The third locus associated with SBP was at 17q21 (rs12946454, MAF 0.28, 0.57 mm Hg higher SBP/minor allele,  $P = 1 \times 10^{-8}$ , Table 2, Figure 1c). This SNP is located in an intron in *PLCD3* (phospholipase C-delta isoform), and is part of a cluster of associated SNPs. *PLCD3* is a member of the phospholipase C family of enzymes; these are important in vascular smooth muscle signaling and are activated by the vasoactive peptides angiotensin II and endothelin<sup>26</sup>. Other genes of interest in the region include: *HEXIM1* and *HEXIM2* (encoding hexamethylene bis-acetamide inducible proteins 1 and 2). Both have been implicated in myocardial growth<sup>27</sup>, cardiac hypertrophy and inflammation<sup>28</sup>.

The DBP SNP with the strongest association evidence on joint analysis is rs1378942 (MAF = 0.36, 0.43 mm Hg higher/minor allele,  $P = 1 \times 10^{-23}$ , Table 2, Figure 1g), which is in an intron of *CSK* at 15q24. This is one of a cluster of associated SNPs spanning ~72kb. Genes in the region include *CYP1A2* (cytochrome P450 enzyme), *CSK* (c-src tyrosine kinase), *LMAN1L* (lectin mannose-binding1 like) and *ARID3b* (encoding AT Rich Interacting Domain protein). Other nearby genes include *CYP1A1* (~60kb) and *CYP11A1* (~418kb). Cytochrome P450 enzymes are responsible for drug and xenobiotic chemical metabolism in the liver and cellular metabolism of arachidonic acid derivatives<sup>29</sup>, some of which influence renal function, peripheral vascular tone and blood pressure. *CYP1A2* is widely expressed, representing 15% of CYP450 enzymes produced in the liver and mediating the metabolism of multiple medications (<http://www.medicine.iupui.edu/Flockhart/table.htm>). A correlated SNP, rs762551 (MAF = 0.31,  $r^2 = 0.63$ , HapMap CEU) in an intron of *CYP1A2* has been found to influence caffeine metabolism and recently association has been suggested between myocardial infarction risk and the allele associated with slow caffeine metabolism<sup>30</sup>. The *ARID3B* gene is embryonic lethal when knocked out in mouse, with branchial arch and vascular developmental abnormalities<sup>31</sup>, but is potentially interesting because of the presence of *ARID5B* at the 10q21 locus described below.

The second DBP SNP is rs16998073 (MAF = 0.21, 0.50 mm Hg higher/minor allele,  $P = 1 \times 10^{-21}$ , Table 2, Figure 1d) which lies 3.4kb upstream of *FGF5* (fibroblast growth factor 5) on 4q21. The FGF5 protein is a member of the fibroblast growth factor (FGF) family that stimulates cell growth and proliferation in multiple cell types, including cardiac myocytes, and has been associated with angiogenesis in the heart<sup>32</sup>.

The third DBP SNP, rs653178 (MAF = 0.47, 0.46 mm Hg lower DBP/major allele,  $P = 3 \times 10^{-18}$ , Table 2, Figure 1f) at 12q24 is in an intron in the *ATXN2* (Ataxin) gene. The SNP is in a cluster of strongly associated SNPs spanning 200kb. This SNP is perfectly correlated with a missense SNP in *SH2B3* (rs3184504, R262W,  $r^2$  in CEU to rs653178 = 1.0, DBP  $P = 3 \times 10^{-7}$  in stage 1 GWAS, change in  $\log_{10}(P) = 0.3$  compared to rs653178). The minor allele of rs3184504, which is associated with higher DBP, has recently been associated with increased odds of type 1 diabetes<sup>33,34</sup>, celiac disease<sup>33,34</sup>, and most recently with eosinophil count, myocardial infarction, with a weak association with hypertension<sup>35</sup>. The *SH2B3* protein (also known as lymphocyte-specific adapter protein, LNK) is one of a subfamily of SH2 domain-containing proteins and is implicated in growth factor, cytokine, and immunoreceptor signaling. In mice, it is primarily expressed in hematopoietic precursor cells, brain, testis and muscle<sup>36</sup>. There is some support for hypertension having an inflammatory component, possibly involving the adaptive immune system<sup>37</sup>. There are no prior studies linking blood pressure with type 1 diabetes or celiac disease. To explore this further, we looked up SNPs reported to be associated with T1D, celiac disease or myocardial infarction in the Global BPgen GWAS results and failed to find convincing association other than that for the *SH2B3* missense SNP (data not shown). It is possible that the *SH2B3* missense SNP impacts blood pressure through an action specific to cells outside of the immune system and that no direct link between blood pressure and autoimmune diseases exists.



The fourth DBP SNP, rs1530440 (MAF = 0.19, 0.39 mm Hg lower/minor allele,  $P = 1 \times 10^{-9}$ , Table 2, Figure 1e) at 10q21 is intronic and one of a cluster of SNPs in *C10orf107*, an open reading frame of unknown function. Nearby genes include *ARID5B* (AT rich interactive domain 5B (MRF1 like)), *TMEM26* (transmembrane protein 26), *RTKN2* (RhoA GTPase effector, rhotekin-2) and *RHOBTB1* (RhoBTB GTPase). The Rho family of GTPases converts guanine triphosphate to inactive guanine diphosphate. The actions of other GTP-modulating enzymes may modulate salt-sensitive hypertension<sup>38,39</sup>. The *ARID5B* gene is a member of the AT-rich interaction domain family of transcription factors and is highly expressed in cardiovascular tissue and involved in smooth muscle cell differentiation<sup>40</sup>.

The fifth DBP SNP, rs16948048 (MAF 0.39, 0.34 mm Hg higher DBP/minor allele,  $P = 5 \times 10^{-9}$ , Table 2, Figure 1h) at 17q21 is upstream of *ZNF652* (zinc finger protein 652) and *PHB* (prohibitin). Neither gene has previously been implicated in hypertension or other cardiovascular phenotypes.

We observed no significant interaction between the eight genome-wide significant SNPs and gender ( $P > 0.01$ , Supplementary Table 5). There was also no evidence of heterogeneity of effect across the samples examined for the eight SNPs (Q-statistic  $P > 0.05$ ).

While we describe here promising candidates at each locus identified, the causal gene could be any of the genes around the association signal in each locus (Figure 1). Fine mapping and resequencing will be required to refine each association signal and to identify likely causal genetic variants which could be studied further in humans and in animal models.

### All variants are related to both blood pressure traits

It remains to be clarified whether SBP or DBP is the better target for genetic investigation of blood pressure. The two traits are correlated and heritable, and both show strong increases with age, with DBP starting to plateau and in some individuals fall at ages above 60–65. Some have advocated the study of pulse pressure (SBP-DBP), which increases with advancing age, and is correlated positively with SBP and negatively with DBP and also shows evidence of heritability. In our GWAS and follow up, we chose *a priori* to consider SBP and DBP as separate traits. Thus, validation was only attempted for either SBP or DBP, according to the trait for which the stage 1  $P$  value was lowest. Because SBP and DBP are correlated ( $r \sim 0.50$ – $0.70$ ), it is perhaps not surprising to see that all eight genome-wide significant SNPs are associated with both SBP and DBP with the same directions of effect (Table 3, Figure 2). Thus, our presentation of results as SBP- or DBP-associated is somewhat arbitrary. The observation that each SNP shows stronger association with one trait or the other (typically by 1–2 orders of magnitude) could reflect sampling variation, small effect sizes or true differences in the underlying biologic basis of one trait or the other. A study designed to examine pulse pressure would be expected to show weaker (if any) association signals for the variants identified which all showed concordant effects on SBP and DBP.

### All variants are related to hypertension

We did not perform a global GWAS of hypertension, which is expected to be underpowered to detect common variants of modest incremental effects on continuous blood pressure. For the eight SNPs that were genome-wide significant in continuous trait analysis, we examined the association with hypertension (SBP  $\geq 140$  mm Hg or DBP  $\geq 90$  mm Hg or antihypertensive medication use) compared to normotension (SBP  $< 120$  mm Hg and DBP  $< 85$  mm Hg and no antihypertensive medication use) in planned secondary analyses (N range = 57,410 – 99,802). All alleles associated with continuous blood pressure were also associated with odds of hypertension in directions consistent with the continuous trait effect (Table 4, Figure 2). The relative yields of the two approaches remain to be fully evaluated and will only become clearer upon completion of large ongoing GWA studies of dichotomous hypertension case-control samples. However, when we examined the hypertension association of each of the 8 SNPs genome-wide significantly associated with continuous SBP or DBP in just the stage 1 Global BPgen samples, 4 had  $0.01 < P \leq 0.10$ . These SNPs would not have been selected for follow-up genotyping had these tests been conducted as part of a hypertension GWAS. Thus, the study of continuous blood pressure allowed us to identify effects on risk of hypertension that would not have been readily discovered in a GWAS of hypertension drawn from these samples.

### Extension to non-European samples

To date, the majority of complex disease association signals reaching genome-wide significance have been concentrated in populations of European ancestry, and it remains unclear whether these findings will transfer to individuals with other genetic backgrounds. We genotyped all stage 2a SNPs (four of which were not confirmed in the European ancestry analyses) in a separate Indian Asian sample of up to 12,889 individuals. We replicated the association of the SNP at 4q21 near *FGF5* (rs16998073,  $P = 5 \times 10^{-4}$ , Supplementary Table 2) and the SNP at 10q24 near *CYP17A1* (rs11191548,  $P = 0.008$ , Supplementary Table 2). We did not replicate association of the SNP rs1378942 at *CYP11A2* ( $P = 0.17$ , same direction), which could reflect limited power to detect the modest effect size, differences in linkage disequilibrium patterns in Indian Asians compared to Europeans, or simply lack of association in individuals of Indian Asian ancestry. The marked allele frequency differences between the European samples (C allele frequency  $\sim 0.35$ ), the Indian Asian samples (0.77) and HapMap YRI (1.00) suggest distinct patterns of genetic variation at this locus across populations. A signal of positive selection has been suggested at the locus41 raising the potential functional importance of genetic variation in the region.

## DISCUSSION

The eight loci described here and the additional loci reported by our colleagues in the CHARGE consortium are among the first confirmed associations between common genetic variants and blood pressure. Each association explains only a very small proportion of the total variation in SBP or DBP ( $\sim 0.05$ – $0.10\%$ , approximately 1 mm Hg/allele SBP or 0.5 mm Hg/allele DBP, Table 2). However, the variants identified here have an aggregate effect on blood pressure, acting throughout the range of values (not just hypertensive), which has been shown to produce meaningful population changes in cardiovascular and stroke risk. For



example, 2 mm Hg lower SBP, across the range of observed values, has been estimated to translate into 6% less stroke and 4% less coronary heart disease.<sup>42</sup>

Given the modest effects observed here and the limited power of this study to detect such effects, it is likely that many more common variants exist with weak effects upon blood pressure. This study illustrates the value of well-powered meta-analysis and follow-up genotyping, accompanied by *in silico* analysis, requiring the coordinated efforts of investigators across multiple studies, to establish definitively the relationship of these loci with blood pressure regulation in the general population.

In a companion paper, the CHARGE consortium reports as genome-wide significant 3 of the 8 loci that reached genome-wide significance in our Global BPgen joint analysis of stages 1+2. CHARGE also reports common variants at 5 additional genome-wide significant loci at: 11p15 (Global BPgen  $P = 0.009$ ), 3p22 ( $P = 0.01$ ), 12q21 ( $P = 0.008$ ), 12q24 ( $P = 0.05$ ), and 10p12 ( $P = 0.004$ , see companion CHARGE paper). While these SNPs did not appear among our top 10 SNPs for either blood pressure trait, the Global BPgen results from *in silico* exchange and for the same alleles are clearly consistent with the conclusions of the CHARGE investigators. Among the 10 SBP and 10 DBP loci at the top of the Global BPgen results, five loci were represented in the CHARGE top 10 results (Supplementary Table 3). With the modest effect sizes we observed, it is not surprising that the top 10 loci for each blood pressure trait would exhibit only partial overlap.

We acknowledge that some limitations apply to our study. The participants in the individual studies comprising Global BPgen and our follow-up cohorts were ascertained using diverse criteria, had their blood pressure measured in a variety of ways, and exhibited a broad range of age and treatment profiles. Even small differences in these factors could reduce power to detect the association of genetic variants with modest effect, although such heterogeneity should not increase the false-positive rate. Even though SBP and DBP are dynamic phenotypes resulting from multiple competing influences, estimates of the test-retest reliability of blood pressure measurements are approximately 0.65–0.75 in studies focused on blood pressure<sup>43,44</sup>. Moreover, a graded relationship between BP measures and cardiovascular risk has been consistently observed, despite variability in BP measures<sup>2</sup>. At the individual level, genetically-determined alteration of 1 mm Hg SBP or 0.5 mm Hg DBP would be difficult to detect in the clinic, but large sample sizes use group-level differences in means to detect small genetic effects.

Exposures such as dietary sodium and potassium intake or excessive alcohol use also contribute to inter-individual differences in blood pressure. These were measured in a minority of our samples and thus we could not meaningfully adjust for these in our study. Under the assumption that these do not alter blood pressure systematically by genotype, we would only expect this omission to reduce power slightly.

We chose *a priori* to adjust for body mass index, which explains ~6–8% of the total variation in SBP and DBP, with the goal of reducing potential non-genetic contributions to blood pressure variability. Genetic variants could influence blood pressure acting through

BMI as an intermediate, but such variants are best identified through BMI GWA studies such as those recently reported by Loos et al<sup>45</sup> and Willer et al<sup>46</sup>.

We adjusted for use of antihypertensive therapy by adding 15 mm Hg and 10 mm Hg to SBP and DBP, respectively. This approach has been shown to be superior to ignoring antihypertensive treatment or to excluding individuals on therapy<sup>13</sup>. However, it is clear that factors such as medication number and dosage, and variation in prescription patterns in different countries and time periods make this adjustment scheme an oversimplification. Again, such effects should generally bias our findings toward the null.

There are many classes of widely used therapies with strong antihypertensive effects. We examined the association of common variants at the loci extending 100kb on either side of the genes encoding the targets for thiazide diuretics (*NCCT*), loop diuretics (*NKCC2*), ACE inhibitors (*ACE*), angiotensin II receptor type 1 blockers (*AGTRI*), beta adrenoreceptor blockers (*ADRB1*, *ADRB2*), alpha adrenoreceptor antagonists (*ADRA1A*, *ADRA1B*, *ADRA1D*), calcium channel blockers (*CACNA1S*, *CACNA1C*, *CACNA1D*, *CACNA1F*), and aldosterone antagonists (*CYP11B2*). No results exceeded chance expectations. This does not exclude the existence of variants of weaker effects or variants that were missed because they were not covered by existing arrays. Obviously, it would be interesting to examine the impact of common variants in these genes on individual responses to therapies, which we have not done.

Moreover, the strength of association of variation in a gene with a trait (or lack thereof) says nothing about the potential strength of a drug designed to agonize or antagonize the product of that gene. For example, a common variant in *HMGCR* has only a modest effect on fasting lipids,<sup>47</sup> yet statin therapy, which inhibits the HMGCR enzyme to lower LDL cholesterol, substantially lowers risk of cardiovascular disease. Thus, the implication of modest common variant genetic effects is not just a function of the ability to identify tendency toward higher or lower blood pressure in carriers of alternate alleles, but also the ability to recognize relevant targets for therapy that have defined *in vivo* relevance in human beings.

While targeted pharmacotherapy has theoretical appeal, clinical trials to demonstrate the utility and cost-effectiveness of such approaches will be required before such personalized medicine could be endorsed. The association signals identified here will need to be refined through fine mapping, and resequencing will be needed to define more fully the allelic spectrum of variants at each locus that contributes to inter-individual differences in blood pressure. Our findings offer initial insights into the genetic basis of a problem of global proportions and the potential for an improved understanding of blood pressure regulation. These loci may point to new targets for blood pressure reduction and ultimately additional opportunities to prevent the growing public health burden of cardiovascular disease.

## METHODS

### Overall study design

An expanded description of the methods is provided in the Supplementary Methods. The study comprised two staged analyses performed separately for SBP and DBP. Stage 1 was a

meta-analysis of directly genotyped and imputed SNPs from individuals of European descent in 17 samples drawn from population-based or control samples in case-control studies in the Global BPgen consortium. In stage 2a, we selected 12 SNPs for genotyping in up to 71,225 individuals of European descent from 13 studies and up to 12,889 individuals of Indian Asian ancestry from one study. In stage 2b, we selected 20 SNPs (10 SBP, 10 DBP) for *in silico* analysis in 29,136 individuals of European descent from the CHARGE consortium (stage 2b, see Supplementary Figure 1).

### Stage 1 samples

The Global BPgen consortium comprises 17 GWAS studies: the Baltimore Longitudinal Study of Aging (BLSA), British 1958 Birth Cohort (B58C-T1DGC and B58C-WTCCC), Cohorte Lausannoise (CoLaus), Diabetes Genetics Initiative (DGI), European Prospective Investigation of Cancer-Norfolk-Genome Wide Association Study (EPIC-Norfolk-GWAS), Fenland Study, Finland-United States Investigation of NIDDM Genetics (FUSION) study, Invecchiare in Chianti (InCHIANTI), Kooperative Gesundheitsforschung in der Region Augsburg (KORA), the Myocardial Infarction Genetics Consortium (MIGen), Northern Finland Birth Cohort of 1966 (NFBC1966), SardiNIA, Study of Health in Pomerania (SHIP), the Precocious Coronary Artery Disease (PROCARDIS), Supplementation en Vitamines et Mineraux Antioxydants (SU.VI.MAX), and TwinsUK. We excluded individuals >70 years of age and individuals ascertained on case status for type 1 or 2 diabetes (DGI, FUSION), coronary artery disease (MIGen, PROCARDIS) or hypertension (BRIGHT), leaving 34,433 individuals for analysis (Table 1). A detailed description of the study design and phenotype measurement for all cohorts can be found in the Supplementary Methods.

### Genome-wide genotyping

Genotyping arrays and quality control filters are provided in Supplementary Table 1.

### Imputation

Imputation of allele dosage of ungenotyped SNPs in HapMap CEU v21a or v22 was performed using MACH48 or IMPUTE49 with parameters and pre-imputation filters as specified in Supplementary Table 1. SNPs were excluded from analysis if the cohort-specific imputation quality as assessed by r2.hat (MACH) or .info (IMPUTE) metrics was <0.30. In total, up to 2,497,993 genotyped or imputed autosomal SNPs were analyzed.

### Phenotype modeling

In individuals taking antihypertensive therapies, blood pressure was imputed by adding 15 mm Hg and 10 mm Hg for SBP and DBP, respectively<sup>13</sup>. Continuous SBP and DBP were adjusted for age, age<sup>2</sup>, body mass index, and any study-specific geographic covariates in gender-specific linear regression models. In FUSION and SardiNIA, which included family-based samples, gender-pooled linear regression was performed with the addition of gender as a covariate. Residuals on the mm Hg scale were used as univariate traits in genotype-phenotype analysis.

In secondary analyses, hypertension was defined by the presence of SBP  $\geq 140$  mm Hg *or* diastolic blood pressure  $\geq 90$  mm Hg *or* self-report of taking a medication for the treatment of hypertension. Normotensive controls were defined as individuals not taking any anti-hypertensives *and* having a SBP  $< 120$  mm Hg *and* a DBP  $< 85$  mm Hg.

### Genotype-phenotype association analysis

Genotype-phenotype association of SBP and DBP residuals was performed under an additive model using software as specified in Supplementary Table 1. Analysis of hypertension for eight genome-wide significant continuous blood pressure loci was performed using logistic regression to adjust for age, age<sup>2</sup>, gender, body mass index.

### Meta-analysis of stage 1 samples

All cohort-specific effect estimates and coded alleles were oriented to the forward strand of the NCBI35 reference sequence of the human genome, using the alphabetically higher allele as the coded allele. For example, for a G/T SNP coded GG=0, GT=1, TT=2, the coded allele would be T. To capture the power loss due to imperfect imputation, we estimated “N effective”, which was the sum of the sample-specific products of the imputation quality metric and the sample size. No filtering on minor allele frequency was used. Genomic control<sup>14</sup> was performed on cohort- and gender-specific test statistics. Lambda estimates are given in Supplementary Table 1; quantile-quantile plots are shown in Supplementary Figure 2. Meta-analysis in stage 1 was performed using inverse variance weights. Stage 1 meta-analysis results were genomic controlled.

### Selection of SNPs for stage 2

12 SNPs were selected for follow-up in stage 2a from among the results with  $P < 10^{-5}$  during interim analyses. For *in silico* exchange with the CHARGE consortium (stage 2b), we identified the top independent loci to select 10 SBP and 10 DBP SNPs. If a SNP in one top 10 list was also among the top 10 for the alternate blood pressure trait, we kept the locus with the lower p-value and went to the next locus on the list for the alternate blood pressure trait. Because a SNP at the 3q26 locus (*MDS1*) was selected in an interim analysis for direct genotyping, it was retained as the tenth locus for DBP even though its significance was reduced in the final stage 1 DBP GWAS analysis.

### Stage 2a samples

We genotyped 12 SNPs in up to 71,225 individuals of European descent from 13 studies – Utrecht Atherosclerosis Risk in Young Adults (ARYA), British Genetics of Hypertension (BRIGHT), EPIC-Italy, EPIC-Norfolk-REP, Finrisk97, FUSION2, London Life Sciences Population (LOLIPOP), Malmö Diet and Cancer-Cardiovascular Cohort (MDC-CC), Metabolic Syndrome in Men (METSIM), Malmo Preventive Project (MPP), The Prevention of Renal and Vascular ENd stage Disease (PREVEND), Prospect-EPIC, and the Utrecht Health Project (UHP) – and in up to 12,889 individuals of Indian Asian ancestry from the LOLIPOP study. Summary demographics are shown in Table 1 and cohort information in the Supplementary Methods).

## Stage 2a follow-up genotyping

For genotyping methods and platforms see Supplementary Methods.

## Stage 2b *in silico* samples

We obtained results based on the analysis of the Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) consortium, which comprises 29,136 samples from five population-based cohorts.

## Pooled analysis of first and second stage samples

Meta-analysis of stage 1, 2a and 2b results was performed using inverse variance weighting. Standard errors were multiplied by the square root of the lambda estimate for genomic control and are presented throughout the text. Nominal *P* values after genomic control<sup>14</sup> are presented. We considered associations genome-wide significant if they exceeded  $P = 5 \times 10^{-8}$ , a Bonferroni correction for the estimated 1M independent common variant tests in the human genome of European-derived individuals<sup>14,15</sup>.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Authors

Christopher Newton-Cheh<sup>1,2,3,94</sup>, Toby Johnson<sup>4,5,6,94</sup>, Vesela Gateva<sup>7,94</sup>, Martin D Tobin<sup>8,94</sup>, Murielle Bochud<sup>5</sup>, Lachlan Coin<sup>9</sup>, Samer S Najjar<sup>10</sup>, Jing Hua Zhao<sup>11,12</sup>, Simon C Heath<sup>13</sup>, Susana Eyheramendy<sup>14,15</sup>, Konstantinos Papadakis<sup>16</sup>, Benjamin F Voight<sup>1,3</sup>, Laura J Scott<sup>7</sup>, Feng Zhang<sup>17</sup>, Martin Farrall<sup>18,19</sup>, Toshiko Tanaka<sup>20,21</sup>, Chris Wallace<sup>22,23</sup>, John C Chambers<sup>9</sup>, Kay-Tee Khaw<sup>12,24</sup>, Peter Nilsson<sup>25</sup>, Pim van der Harst<sup>26</sup>, Silvia Polidoro<sup>27</sup>, Diederick E Grobbee<sup>28</sup>, N Charlotte Onland-Moret<sup>28,29</sup>, Michiel L Bots<sup>28</sup>, Louise V Wain<sup>8</sup>, Katherine S Elliott<sup>19</sup>, Alexander Teumer<sup>30</sup>, Jian'an Luan<sup>11</sup>, Gavin Lucas<sup>31</sup>, Johanna Kuusisto<sup>32</sup>, Paul R Burton<sup>8</sup>, David Hadley<sup>16</sup>, Wendy L McArdle<sup>33</sup>, Wellcome Trust Case Control Consortium<sup>34</sup>, Morris Brown<sup>35</sup>, Anna Dominiczak<sup>36</sup>, Stephen J Newhouse<sup>22</sup>, Nilesh J Samani<sup>37</sup>, John Webster<sup>38</sup>, Eleftheria Zeggini<sup>19,39</sup>, Jacques S Beckmann<sup>4,40</sup>, Sven Bergmann<sup>4,6</sup>, Noha Lim<sup>41</sup>, Kijoung Song<sup>41</sup>, Peter Vollenweider<sup>42</sup>, Gerard Waeber<sup>42</sup>, Dawn M Waterworth<sup>41</sup>, Xin Yuan<sup>41</sup>, Leif Groop<sup>43,44</sup>, Marju Orho-Melander<sup>25</sup>, Alessandra Allione<sup>27</sup>, Alessandra Di Gregorio<sup>27,45</sup>, Simonetta Guarrera<sup>27</sup>, Salvatore Panico<sup>46</sup>, Fulvio Ricceri<sup>27</sup>, Valeria Romanazzi<sup>27,45</sup>, Carlotta Sacerdote<sup>47</sup>, Paolo Vineis<sup>9,27</sup>, Inês Barroso<sup>12,39</sup>, Manjinder S Sandhu<sup>11,12,24</sup>, Robert N Luben<sup>12,24</sup>, Gabriel J. Crawford<sup>3</sup>, Pekka Jousilahti<sup>48</sup>, Markus Perola<sup>48,49</sup>, Michael Boehnke<sup>7</sup>, Lori L Bonnycastle<sup>50</sup>, Francis S Collins<sup>50</sup>, Anne U Jackson<sup>7</sup>, Karen L Mohlke<sup>51</sup>, Heather M Stringham<sup>7</sup>, Timo T Valle<sup>52</sup>, Cristen J Willer<sup>7</sup>, Richard N Bergman<sup>53</sup>, Mario A Morken<sup>50</sup>, Angela Döring<sup>15</sup>, Christian Gieger<sup>15</sup>, Thomas Illig<sup>15</sup>, Thomas Meitinger<sup>54,55</sup>, Elin Org<sup>56</sup>, Arne Pfeufer<sup>54</sup>, H Erich Wichmann<sup>15,57</sup>, Sekar Kathiresan<sup>1,2,3</sup>, Jaume Marrugat<sup>31</sup>, Christopher J O'Donnell<sup>58,59</sup>, Stephen M Schwartz<sup>60,61</sup>, David S Siscovick<sup>60,61</sup>, Isaac Subirana<sup>31,62</sup>, Nelson B Freimer<sup>63</sup>,

Anna-Liisa Hartikainen<sup>64</sup>, Mark I McCarthy<sup>19,65,66</sup>, Paul F O'Reilly<sup>9</sup>, Leena Peltonen<sup>39,49</sup>, Anneli Pouta<sup>64,67</sup>, Paul E de Jong<sup>68</sup>, Harold Snieder<sup>69</sup>, Wiek H van Gilst<sup>26</sup>, Robert Clarke<sup>70</sup>, Anuj Goel<sup>18,19</sup>, Anders Hamsten<sup>71</sup>, John F Peden<sup>18,19</sup>, Udo Seedorf<sup>72</sup>, Ann-Christine Syvänen<sup>73</sup>, Giovanni Tognoni<sup>74</sup>, Edward G Lakatta<sup>10</sup>, Serena Sanna<sup>75</sup>, Paul Scheet<sup>76</sup>, David Schlessinger<sup>77</sup>, Angelo Scuteri<sup>78</sup>, Marcus Dörr<sup>79</sup>, Florian Ernst<sup>30</sup>, Stephan B Felix<sup>79</sup>, Georg Homuth<sup>30</sup>, Roberto Lorbeer<sup>80</sup>, Thorsten Reffeltmann<sup>79</sup>, Rainer Rettig<sup>81</sup>, Uwe Völker<sup>30</sup>, Pilar Galan<sup>82</sup>, Ivo G Gut<sup>13</sup>, Serge Hercberg<sup>82</sup>, G Mark Lathrop<sup>13</sup>, Diana Zeleneka<sup>13</sup>, Panos Deloukas<sup>12,39</sup>, Nicole Soranzo<sup>17,39</sup>, Frances M Williams<sup>17</sup>, Guangju Zhai<sup>17</sup>, Veikko Salomaa<sup>48</sup>, Markku Laakso<sup>32</sup>, Roberto Elosua<sup>31,62</sup>, Nita G Forouhi<sup>11</sup>, Henry Völzke<sup>80</sup>, Cuno S Uiterwaal<sup>28</sup>, Yvonne T van der Schouw<sup>28</sup>, Mattijs E Numans<sup>28</sup>, Giuseppe Matullo<sup>27,45</sup>, Gerjan Navis<sup>68</sup>, Göran Berglund<sup>25</sup>, Sheila A Bingham<sup>12,83</sup>, Jaspal S Kooner<sup>84</sup>, Andrew D Paterson<sup>85</sup>, John M Connell<sup>36</sup>, Stefania Bandinelli<sup>86</sup>, Luigi Ferrucci<sup>21</sup>, Hugh Watkins<sup>18,19</sup>, Tim D Spector<sup>17</sup>, Jaakko Tuomilehto<sup>52,87,88</sup>, David Altshuler<sup>1,3,89,90</sup>, David P Strachan<sup>16</sup>, Maris Laan<sup>56</sup>, Pierre Meneton<sup>91</sup>, Nicholas J Wareham<sup>11,12</sup>, Manuela Uda<sup>75</sup>, Marjo-Riitta Jarvelin<sup>9,67,92</sup>, Vincent Mooser<sup>41</sup>, Olle Melander<sup>25</sup>, Ruth JF Loos<sup>11,12</sup>, Paul Elliott<sup>9,95</sup>, Goncalo R Abecasis<sup>93,95</sup>, Mark Caulfield<sup>22,95</sup>, and Patricia B Munroe<sup>22,95</sup>

## Affiliations

<sup>1</sup> Center for Human Genetic Research, Massachusetts General Hospital, 185 Cambridge Street, Boston, MA 02114, USA <sup>2</sup> Cardiovascular Research Center, Massachusetts General Hospital, Boston, Massachusetts 02114, USA <sup>3</sup> Program in Medical and Population Genetics, Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, Massachusetts, 02142, USA <sup>4</sup> Department of Medical Genetics, University of Lausanne, 1005 Lausanne, Switzerland <sup>5</sup> University Institute for Social and Preventative Medicine, Centre Hospitalier Universitaire Vaudois (CHUV) and University of Lausanne, 1005 Lausanne, Switzerland <sup>6</sup> Swiss Institute of Bioinformatics, Switzerland <sup>7</sup> Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI 48109, USA <sup>8</sup> Departments of Health Sciences & Genetics, Adrian Building, University of Leicester, University Road, Leicester LE1 7RH <sup>9</sup> Department of Epidemiology and Public Health, Imperial College London, St Mary's Campus, Norfolk Place, London W2 1PG, UK <sup>10</sup> Laboratory of Cardiovascular Science, Intramural Research Program, National Institute on Aging, National Institutes of Health, Baltimore, Maryland, USA 21224 <sup>11</sup> MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK <sup>12</sup> Cambridge - Genetics of Energy Metabolism (GEM) Consortium, Cambridge, UK <sup>13</sup> Centre National de Génotypage, 2 rue Gaston Crémieux, CP 5721, 91 057 Evry Cedex, France <sup>14</sup> Pontificia Universidad Católica de Chile, Vicuña Mackenna 4860, Facultad de Matemáticas, Casilla 306, Santiago 22, Chile, 7820436 <sup>15</sup> Institute of Epidemiology, Helmholtz Zentrum München, German Research Centre for Environmental Health, 85764 Neuherberg, Germany <sup>16</sup> Division of Community Health Sciences, St George's, University of London, London SW17 0RE, UK <sup>17</sup> Dept of Twin Research & Genetic Epidemiology, King's College London, London SE1 7EH <sup>18</sup> Dept.



Cardiovascular Medicine, University of Oxford <sup>19</sup> The Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford, OX3 7BN, UK <sup>20</sup> Medstar Research Institute, 3001 S. Hanover Street, Baltimore, MD 21250, USA <sup>21</sup> Clinical Research Branch, National Institute on Aging, Baltimore, MD, 21250 USA <sup>22</sup> Clinical Pharmacology and The Genome Centre, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London EC1M 6BQ <sup>23</sup> JDRF/WT Diabetes and Inflammation Laboratory, Cambridge Institute for Medical Research University of Cambridge, Wellcome Trust/MRC Building, Addenbrooke's Hospital Cambridge, CB2 0XY <sup>24</sup> Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge, Cambridge CB2 2SR, UK <sup>25</sup> Department of Clinical Sciences, Lund University, Malmö University Hospital, SE-20502 Malmö, Sweden <sup>26</sup> Department of Cardiology University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands <sup>27</sup> ISI Foundation (Institute for Scientific Interchange), Villa Gualino, Torino, 10133, Italy <sup>28</sup> Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, STR 6.131, PO Box 85500, 3508 GA Utrecht, The Netherlands <sup>29</sup> Complex Genetics Section, Department of Medical Genetics - DBG, University Medical Center Utrecht, STR 2.2112, PO Box 85500, 3508 GA Utrecht, The Netherlands <sup>30</sup> Interfaculty Institute for Genetics and Functional Genomics, Ernst-Moritz-Arndt-University Greifswald, 17487 Greifswald, Germany <sup>31</sup> Cardiovascular Epidemiology and Genetics, Institut Municipal d'Investigació Mèdica, Barcelona, Spain <sup>32</sup> Department of Medicine University of Kuopio 70210 Kuopio, Finland <sup>33</sup> ALSPAC Laboratory, Department of Social Medicine, University of Bristol, BS8 2BN, UK <sup>34</sup> A full list of authors is provided in the supplementary methods online <sup>35</sup> Clinical Pharmacology Unit, University of Cambridge, Addenbrookes Hospital, Cambridge, UK CB2 2QQ <sup>36</sup> BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, UK G12 8TA <sup>37</sup> Dept of Cardiovascular Science, University of Leicester, Glenfield Hospital, Groby Road, Leicester, LE3 9QP, UK <sup>38</sup> Aberdeen Royal Infirmary, Aberdeen, UK <sup>39</sup> Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK <sup>40</sup> Service of Medical Genetics, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, 1011, Switzerland <sup>41</sup> Genetics Division, GlaxoSmithKline, King of Prussia, PA 19406, USA <sup>42</sup> Department of Internal Medicine, Centre Hospitalier Universitaire Vaudois (CHUV) 1011 Lausanne, Switzerland <sup>43</sup> Department of Clinical Sciences, Diabetes and Endocrinology Research Unit, University Hospital, Malmö <sup>44</sup> Lund University, Malmö S-205 02, Sweden <sup>45</sup> Department of Genetics, Biology and Biochemistry, University of Torino, Torino, 10126, Italy <sup>46</sup> Department of Clinical and Experimental Medicine, Federico II University, Naples, 80100, Italy <sup>47</sup> Unit of Cancer Epidemiology, University of Turin and Centre for Cancer Epidemiology and Prevention (CPO Piemonte), Turin, 10126, Italy <sup>48</sup> National Institute for Welfare and Health P.O. Box 30, FI-00271 Helsinki, Finland <sup>49</sup> Institute for Molecular Medicine Finland FIMM, University of Helsinki and National Public Health Institute <sup>50</sup> Genome Technology Branch, National Human Genome Research Institute, Bethesda, MD 20892, USA <sup>51</sup>

Department of Genetics, University of North Carolina, Chapel Hill, NC 27599, USA  
<sup>52</sup> Diabetes Unit, Department of Epidemiology and Health Promotion, National Public Health Institute, 00300 Helsinki, Finland <sup>53</sup> Physiology and Biophysics USC School of Medicine 1333 San Pablo Street, MMR 626 Los Angeles, California 90033 <sup>54</sup> Institute of Human Genetics, Helmholtz Zentrum Munchen, German Research Centre for Environmental Health, 85764 Neuherberg, Germany <sup>55</sup> Institute of Human Genetics, Technische Universität München, 81675 Munich, Germany <sup>56</sup> Institute of Molecular and Cell Biology, University of Tartu, 51010 Tartu, Estonia <sup>57</sup> Ludwig Maximilians University, IBE, Chair of Epidemiology, Munich <sup>58</sup> Cardiovascular Research Center and Cardiology Division, Massachusetts General Hospital, Boston, Massachusetts 02114, USA <sup>59</sup> Framingham Heart Study and National, Heart, Lung, and Blood Institute, Framingham, Massachusetts 01702, USA <sup>60</sup> Cardiovascular Health Research Unit, Departments of Medicine and Epidemiology, University of Washington, Seattle, Washington, 98101 USA <sup>61</sup> Department of Epidemiology, University of Washington, Seattle, Washington, 98195 USA <sup>62</sup> CIBER Epidemiología y Salud Pública, Barcelona, Spain <sup>63</sup> Center for Neurobehavioral Genetics, Gonda Center, Room 3506, 695 Charles E Young Drive South, Box 951761, UCLA, Los Angeles, CA 90095 <sup>64</sup> Department of Clinical Sciences/Obstetrics and Gynecology, P.O. Box 5000 Fin-90014, University of Oulu, Finland <sup>65</sup> Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Old Road, Headington, Oxford OX3 7LJ, UK <sup>66</sup> Oxford NIHR Biomedical Research Centre, Churchill Hospital, Old Road, Headington, Oxford, UK OX3 7LJ <sup>67</sup> Department of Child and Adolescent Health, National Public Health Institute (KTL), Aapistie 1, P.O. Box 310, FIN-90101 Oulu, Finland <sup>68</sup> Division of Nephrology, Department of Medicine University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands <sup>69</sup> Unit of Genetic Epidemiology and Bioinformatics, Department of Epidemiology University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands <sup>70</sup> Clinical Trial Service Unit and Epidemiological Studies Unit (CTSU), University of Oxford, Richard Doll Building, Roosevelt Drive, Oxford, OX3 7LF, UK <sup>71</sup> Atherosclerosis Research Unit, Department of Medicine Solna, Karolinska Institutet, Karolinska University Hospital Solna, Building L8:03, S-17176 Stockholm, Sweden <sup>72</sup> Leibniz-Institut für Arterioskleroseforschung an der Universität Münster, Domagkstr. 3, D-48149, Münster, Germany <sup>73</sup> Molecular Medicine, Dept. Medical Sciences, Uppsala University, SE-751 85 Uppsala, Sweden <sup>74</sup> Consorzio Mario Negri Sud, Via Nazionale, 66030 Santa Maria Imbaro (Chieti), Italy <sup>75</sup> Istituto di Neurogenetica e Neurofarmacologia, CNR, Monserrato, 09042 Cagliari, Italy <sup>76</sup> Department of Epidemiology, Univ. of Texas M. D. Anderson Cancer Center, Houston, TX 77030 <sup>77</sup> Laboratory of Genetics, Intramural Research Program, National Institute on Aging, National Institutes of Health, Baltimore, Maryland, USA 21224 <sup>78</sup> Unità Operativa Geriatria, Istituto Nazionale Ricovero e Cura per Anziani (INRCA) IRCCS, Rome, Italy <sup>79</sup> Department of Internal Medicine B, Ernst-Moritz-Arndt-University Greifswald, 17487 Greifswald, Germany <sup>80</sup> Institute for Community Medicine, Ernst-Moritz-

Arndt-University Greifswald, 17487 Greifswald, Germany <sup>81</sup> Institute of Physiology, Ernst-Moritz-Arndt-University Greifswald, 17487 Greifswald, Germany <sup>82</sup> U557 Institut National de la Sante et de la Recherche Médicale, U1125 Institut National de la Recherche Agronomique, Université Paris 13, 74 rue Marcel Cachin, 93017 Bobigny Cedex, France <sup>83</sup> MRC Dunn Human Nutrition Unit, Wellcome Trust/MRC Building, Cambridge CB2 0XY, U.K <sup>84</sup> National Heart and Lung Institute, Imperial College London SW7 2AZ <sup>85</sup> Program in Genetics and Genome Biology, Hospital for Sick Children, Toronto, Canada, Dalla Lana School of Public Health, University of Toronto, Toronto, Canada M5T 3M7 <sup>86</sup> Geriatric Rehabilitation Unit, Azienda Sanitaria Firenze (ASF), 50125, Florence, Italy <sup>87</sup> Department of Public Health, University of Helsinki, 00014 Helsinki, Finland <sup>88</sup> South Ostrobothnia Central Hospital, 60220 Seinäjoki, Finland <sup>89</sup> Department of Medicine and Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA <sup>90</sup> Diabetes Unit, Massachusetts General Hospital, Boston, Massachusetts 02114, USA <sup>91</sup> U872 Institut National de la Santé et de la Recherche Médicale, Faculté de Médecine Paris Descartes, 15 rue de l'Ecole de Médecine, 75270 Paris Cedex, France <sup>92</sup> Institute of Health Sciences and Biocenter Oulu, Aapistie 1, FIN-90101, University of Oulu, Finland <sup>93</sup> Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, Michigan 48109 USA

## Acknowledgments

The authors would like to thank the many colleagues who contributed to collection and phenotypic characterization of the clinical samples, as well as genotyping and analysis of the GWA data. They would also especially like to thank those who agreed to participate in the studies. Major funding for the work described in the paper comes from (alphabetically): Academy of Finland (124243, 129322, 129494, 118065), AGAUR (SGR 2005/00577), Albert Pahlsson Research Foundation, Alexander-von-Humboldt Foundation (V-Fokoop-1113183), American Diabetes Association, AstraZeneca AB, AVIS Torino blood donor organization, Barts and The London Charity, Biocenter of University of Oulu, Board of the UMC Utrecht, British Heart Foundation (PG02/128, FS/05/061/19501, SP/04/002), Burroughs Wellcome Fund, CamStrad, Cancer Research United Kingdom, CIBER Epidemiología y Salud Pública, Commissariat à l'Energie Atomique, Compagnia di San Paolo to the ISI Foundation (Torino, Italy), Conservatoire National des Arts et Métiers, Crafoord Foundation, Donovan Family Foundation, Doris Duke Charitable Foundation, Dutch Kidney Foundation (E033), Dutch College of Healthcare Insurance Companies, Dutch Ministry of Health, Dutch Organisation of Health Care Research, ENGAGE (HEALTH-F4-2007-201413), Ernhold Lundströms Research Foundation, Estonian Ministry of Education and Science (0182721s06), EURO-BLCS, European Commission (QLG1-CT-2000-01643, LSHM-CT-2007-037273), European Commission-Europe Against Cancer (AEP/90/05), European Union (FP-6 LSHM-CT-2003-503041, FP-6 LSHM CT 2006 037697), European Society for the Study of Diabetes, Faculty of Biology and Medicine of Lausanne, Switzerland, Fannie E. Rippel Foundation, Finnish Foundation for Cardiovascular Research, FIS (CP05/00290), German Federal Ministry of Education and Research (01ZZ9603, 01ZZ0103, 01ZZ0403, 03ZIK012, 01EZ0874), German National Genome Research Network, German Research Center for Environmental Health, (Neuherberg, Germany), Giorgi-Cavaglieri Foundation, GlaxoSmithKline, Guy's & St Thomas' NHS Foundation Trust, Health Research and Development Council of the Netherlands (2100.0008, 2100.0042), Helmholtz Zentrum München, Hulda and Conrad Mossfelt Foundation, Institut National de la Recherche Agronomique, Institut National de la Sante et de la Recherche Médicale, Italian Association for Research on Cancer, Italian Ministry of Health (110.1RS97.71), Italian National Research Council, Juvenile Diabetes Research Fund, King Gustaf V and Queen Victoria Foundation, King's College London and King's College Hospital NHS Foundation Trust, Knut and Alice Wallenberg Foundation, Lennart Hanssons Memorial Fund, LK Research Funds, Massachusetts General Hospital Cardiovascular Research Center and Department of Medicine, Medical Faculty of Lund University and Malmö University Hospital, Medical Research Council of the UK (G0000934, G0501942, G9521010D), Medical Research Council-GlaxoSmithKline (85374), MedStar Research Institute, Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III (RD06/0009), Ministry of Cultural Affairs and Social Ministry (Federal State of Mecklenburg-West Pomerania), National Institute for Health Research (NIHR), National Institute for Health Research Cambridge Biomedical Research Centre, Novartis Institute for Biomedical Research, NWO VENI (916.76.170), Province of Utrecht, Region Skane, Siemens Healthcare (Erlangen, Germany), Sigrid Juselius Foundation, Stockholm County Council (562183), Support for

Science Funding programme, Swedish Heart and Lung Foundation, Swedish Medical Research Council, Swedish National Research Council, Swedish Research Council (8691), Swiss National Science Foundation (33CSO-122661, 310030-112552, 3100AO-116323/1, PROSPER 3200BO-111362/1, 3233BO-111361/1), UNIL, University of Utrecht, US National Institutes of Health (U01DK062418, K23HL80025, DK062370, DK072193, U54DA021519, 1Z01HG000024, N01AG-916413, N01AG-821336, 263MD916413, 263MD821336, Intramural NIA, R01HL087676, K23HL083102, U54RR020278, R01HL056931, P30ES007033, R01HL087679, RL1MH083268, 263-MA-410953, N01-AG-1-2109, N01-HD-1-3107), WCRF (98A04, 2000/30), Wellcome Trust (068545/Z/02, 076113/B/04/Z, 079895, 070191/Z/03/Z, 077016/Z/05/Z, WT088885/Z/09/Z).

## References

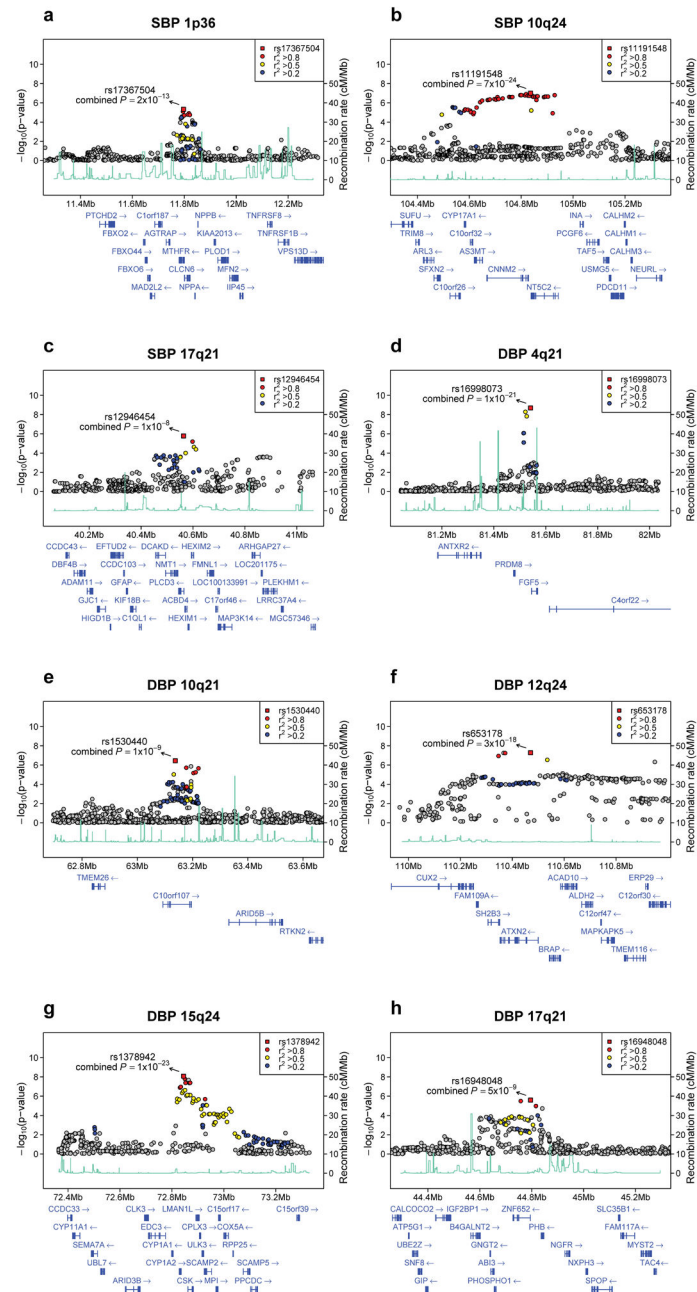
1. Ezzati M, Lopez AD, Rodgers A, Vander Hoorn S, Murray CJ. Selected major risk factors and global and regional burden of disease. *Lancet*. 2002; 360:1347–60. [PubMed: 12423980]
2. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*. 2002; 360:1903–1913. [PubMed: 12493255]
3. The world health report 2002. Reducing risks, promoting healthy life. World Health Organization; 2002.
4. Whelton PK, et al. Primary prevention of hypertension: clinical and public health advisory from The National High Blood Pressure Education Program. *Jama*. 2002; 288:1882–8. [PubMed: 12377087]
5. Havlik RJ, et al. Blood pressure aggregation in families. *Am J Epidemiol*. 1979; 110:304–12. [PubMed: 474567]
6. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. *Cell*. 2001; 104:545–556. [PubMed: 11239411]
7. Ji W, et al. Rare independent mutations in renal salt handling genes contribute to blood pressure variation. *Nat Genet*. 2008; 40:592–9. [PubMed: 18391953]
8. Newhouse SJ, et al. Haplotypes of the WNK1 gene associate with blood pressure variation in a severely hypertensive population from the British Genetics of Hypertension study. *Hum Mol Genet*. 2005; 14:1805–14. [PubMed: 15888480]
9. Tobin MD, et al. Association of WNK1 gene polymorphisms and haplotypes with ambulatory blood pressure in the general population. *Circulation*. 2005; 112:3423–9. [PubMed: 16301342]
10. Tobin MD, et al. Common variants in genes underlying monogenic hypertension and hypotension and blood pressure in the general population. *Hypertension*. 2008; 51:1658–64. [PubMed: 18443236]
11. Genome-wide association study of 14 000 cases of seven common diseases and 3,000 shared controls. *Nature*. 2007; 447:661–678. [PubMed: 17554300]
12. Levy D, et al. Framingham Heart Study 100K Project: genome-wide associations for blood pressure and arterial stiffness. *BMC Med Genet*. 2007; 8(Suppl 1):S3. [PubMed: 17903302]
13. Tobin MD, Sheehan NA, Scurrah KJ, Burton PR. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. *Stat Med*. 2005; 24:2911–35. [PubMed: 16152135]
14. Devlin B, Roeder K. Genomic control for association studies. *Biometrics*. 1999; 55:997–1004. [PubMed: 11315092]
15. Pe'er I, Yelensky R, Altshuler D, Daly MJ. Estimation of the multiple testing burden for genomewide association studies of nearly all common variants. *Genet Epidemiol*. 2008; 32:381–385. [PubMed: 18348202]
16. Martin RM, et al. P450c17 deficiency in Brazilian patients: biochemical diagnosis through progesterone levels confirmed by CYP17 genotyping. *J Clin Endocrinol Metab*. 2003; 88:5739–46. [PubMed: 14671162]
17. Geller DH, Auchus RJ, Mendonca BB, Miller WL. The genetic and functional basis of isolated 17,20-lyase deficiency. *Nat Genet*. 1997; 17:201–5. [PubMed: 9326943]
18. Kluijtmans LA, et al. Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. *Am J Hum Genet*. 1996; 58:35–41. [PubMed: 8554066]

19. Sohda S, et al. Methylene tetrahydrofolate reductase polymorphism and pre-eclampsia. *J Med Genet.* 1997; 34:525–6. [PubMed: 9192280]
20. Qian X, Lu Z, Tan M, Liu H, Lu D. A meta-analysis of association between C677T polymorphism in the methylene tetrahydrofolate reductase gene and hypertension. *Eur J Hum Genet.* 2007; 15:1239–45. [PubMed: 17726486]
21. John SW, et al. Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension. *Science.* 1995; 267:679–681. [PubMed: 7839143]
22. Newton-Cheh C, et al. Association of Common Variants in NPPA and NPPB with Circulating Natriuretic Peptides and Blood Pressure. *Nature Genetics.* 2009 In Press.
23. Simon DB, et al. Genetic heterogeneity of Bartter's syndrome revealed by mutations in the K<sup>+</sup> channel, ROMK. *Nat Genet.* 1996; 14:152–6. [PubMed: 8841184]
24. Simon DB, et al. Gitelman's variant of Bartter's syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter. *Nat Genet.* 1996; 12:24–30. [PubMed: 8528245]
25. Daviet L, et al. Cloning and characterization of ATRAP, a novel protein that interacts with the angiotensin II type 1 receptor. *J Biol Chem.* 1999; 274:17058–62. [PubMed: 10358057]
26. Suh PG, et al. Multiple roles of phosphoinositide-specific phospholipase C isozymes. *BMB Rep.* 2008; 41:415–34. [PubMed: 18593525]
27. Montano MM, et al. Mutation of the HEXIM1 gene results in defects during heart and vascular development partly through downregulation of vascular endothelial growth factor. *Circ Res.* 2008; 102:415–22. [PubMed: 18079413]
28. Dey A, Chao SH, Lane DP. HEXIM1 and the control of transcription elongation: from cancer and inflammation to AIDS and cardiac hypertrophy. *Cell Cycle.* 2007; 6:1856–63. [PubMed: 17671421]
29. Nebert DW, Dalton TP. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. *Nat Rev Cancer.* 2006; 6:947–60. [PubMed: 17128211]
30. Cornelis MC, El-Sohemy A, Kabagambe EK, Campos H. Coffee, CYP1A2 genotype, and risk of myocardial infarction. *JAMA.* 2006; 295:1135–41. [PubMed: 16522833]
31. Takebe A, et al. Microarray analysis of PDGFR alpha<sup>+</sup> populations in ES cell differentiation culture identifies genes involved in differentiation of mesoderm and mesenchyme including ARID3b that is essential for development of embryonic mesenchymal cells. *Dev Biol.* 2006; 293:25–37. [PubMed: 16530748]
32. Vatner SF. FGF induces hypertrophy and angiogenesis in hibernating myocardium. *Circ Res.* 2005; 96:705–7. [PubMed: 15831820]
33. Todd JA, et al. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat Genet.* 2007; 39:857–64. [PubMed: 17554260]
34. Hunt KA, et al. Newly identified genetic risk variants for celiac disease related to the immune response. *Nat Genet.* 2008; 40:395–402. [PubMed: 18311140]
35. Gudbjartsson DF, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet.* 2009
36. Velazquez L, et al. Cytokine signaling and hematopoietic homeostasis are disrupted in Lnk-deficient mice. *J Exp Med.* 2002; 195:1599–611. [PubMed: 12070287]
37. Rodriguez-Iturbe B, Vaziri ND, Herrera-Acosta J, Johnson RJ. Oxidative stress, renal infiltration of immune cells, and salt-sensitive hypertension: all for one and one for all. *Am J Physiol Renal Physiol.* 2004; 286:F606–16. [PubMed: 15001451]
38. Du YH, Guan YY, Alp NJ, Channon KM, Chen AF. Endothelium-specific GTP cyclohydrolase I overexpression attenuates blood pressure progression in salt-sensitive low-renin hypertension. *Circulation.* 2008; 117:1045–54. [PubMed: 18268143]
39. Zheng JS, et al. Gene transfer of human guanosine 5'-triphosphate cyclohydrolase I restores vascular tetrahydrobiopterin level and endothelial function in low renin hypertension. *Circulation.* 2003; 108:1238–45. [PubMed: 12925450]
40. Watanabe M, et al. Regulation of smooth muscle cell differentiation by AT-rich interaction domain transcription factors Mrf2alpha and Mrf2beta. *Circ Res.* 2002; 91:382–9. [PubMed: 12215486]

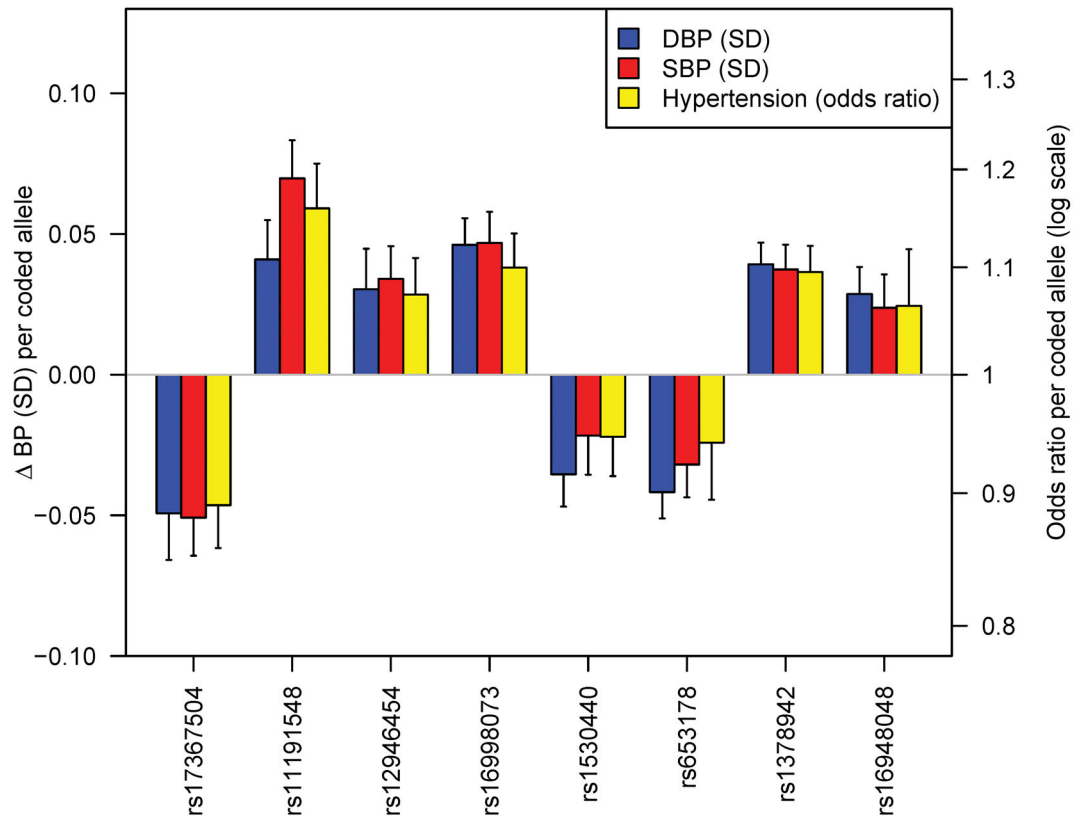


41. Voight BF, Kudaravalli S, Wen X, Pritchard JK. A map of recent positive selection in the human genome. *PLoS Biol.* 2006; 4:e72. [PubMed: 16494531]
42. Stamler J, et al. INTERSALT study findings. Public health and medical care implications. *Hypertension.* 1989; 14:570–7. [PubMed: 2807518]
43. Dyer AR, Shipley M, Elliott P. Urinary electrolyte excretion in 24 hours and blood pressure in the INTERSALT Study. I. Estimates of reliability. The INTERSALT Cooperative Research Group. *Am J Epidemiol.* 1994; 139:927–39. [PubMed: 8166143]
44. Variability of blood pressure and the results of screening in the hypertension detection and follow-up program. *J Chronic Dis.* 1978; 31:651–67. [PubMed: 730822]
45. Loos RJ, et al. Common variants near MC4R are associated with fat mass, weight and risk of obesity. *Nat Genet.* 2008; 40:768–75. [PubMed: 18454148]
46. Willer CJ, et al. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet.* 2009; 41:25–34. [PubMed: 19079261]
47. Kathiresan S, et al. Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. *Nat Genet.* 2008; 40:189–197. [PubMed: 18193044]
48. Li Y, Abecasis GR. Mach 1.0: Rapid haplotype reconstruction and missing genotype inference. *Am J Hum Genet.* 2006; S79:2290.
49. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet.* 2007; 39:906–913. [PubMed: 17572673]
50. Myers S, Bottolo L, Freeman C, McVean G, Donnelly P. A fine-scale map of recombination rates and hotspots across the human genome. *Science.* 2005; 310:321–4. [PubMed: 16224025]



**Figure 1.**

Regional association plots of eight blood pressure loci. For each locus, we show the region extending to within 500kb of a SNP with  $P < 10^{-4}$  on either side. Statistical significance of associated SNPs at each locus are illustrated on the  $-\log_{10}(P)$  scale as a function of chromosomal position (NCBI Build 35). The sentinel SNP at each locus is shown in red. The correlation of the sentinel SNP to other SNPs at the locus is shown on a scale from minimal (gray and blue), to maximal (red). The meta-analysis result for stage 1 is shown with a red square. The joint analysis result (combined  $P$ ) for stage 1+2a+2b is shown with an arrow. Fine-scale recombination rate from Myers et al50 is plotted in aqua.



**Figure 2.**

Relationship of genome-wide significant loci to SBP, DBP and hypertension. Shown are the effects of each variant on continuous SBP and DBP and on the odds ratio for dichotomous hypertension compared to normotension (see **Methods**). For comparability, SBP and DBP effects are shown on the standard deviation scale (SBP SD = 16.6 mm Hg, DBP SD = 10.9 mm Hg). Alleles are coded as shown in Table 2.

**Table 1**

**Study sample characteristics**

Study characteristics are shown for cohort samples examined in stage 1 meta-analysis (population-based and controls from case-control studies), stage 2a (direct genotyping follow-up) and stage 2b (*in silico* follow-up with the CHARGE consortium). Population Cohorts: The Baltimore Longitudinal Study of Aging (BLSA), British 1958 Birth Cohort- Wellcome Trust Case Control Consortium (B58C-WTCCC), British 1958 Birth Cohort – Type 1 Diabetes Genetics Consortium (B58C-T1DGC), Cohorte Lausannoise (CoLaus), European Prospective Investigation of Cancer- Norfolk-Genome Wide Association Study (EPIC-Norfolk-GWAS), Fenland Study (Fenland), Invecchiare in Chianti (InCHIANTI), Kooperative Gesundheitsforschung in der Region Augsburg (KORA), Northern Finland Birth Cohort of 1966 (NFBC1966), SardinIA, Study of Health in Pomerania (SHIP), Supplementation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) and TwinsUK. Controls from case-control studies: Diabetes Genetics Initiative (DGI), Finland-United States Investigation of NIDDM Genetics (FUSION), the Myocardial Infarction Genetics Consortium (MIGen), the Precocious Coronary Artery Disease (PROCARDIS) study. Direct genotyping: The Utrecht Atherosclerosis Risk in Young Adults (AYRA), British Genetics of Hypertension study – hypertension cases (BRIGHT-HTN), BRIGHT study normotensive controls (BRIGHT-NT), EPIC-Italy, EPIC-Norfolk-Replication cohort (EPIC-Norfolk-REP), Finrisk97, FUSION stage 2 controls (FUSION2), London Life Sciences Population (LOLIPOP), Malmö Diet and Cancer Cardiovascular Cohort (MDC), Malmö Preventive Project (MPP), Prevention of Renal and Vascular End stage Disease (PREVEND), Metabolic Syndrome in Men Study (METSIM), Prospect-EPIC cohort, Utrecht Health Project (UHP). NA = not available. HTN = hypertension

Study	N	% women	Age (SD) years	SBP (SD) mm Hg	DBP (SD) mm Hg	BMI (SD) kg/m2	% HTN <sup>#</sup>	% anti-hypertensive therapy
<b>Stage 1 – GWAS</b>								
<b>Population-based cohorts</b>								
BLSA	708	44	42.4 (13.2)	119.5 (15.0)	77.3 (10.2)	24.5 (3.6)	23.2	5.2
B58C – T1DGC*	2,580	51	44.3 (0.3)	121.7 (15.3)	79.4 (10.5)	27.4 (4.9)	20.5	4.7
B58C – WTCCC*	1,473	50	44.9 (0.4)	126.7 (15.2)	79.1 (10.2)	27.4 (4.7)	17.4	4.2
CoLaus	4,969	53	51.7 (9.5)	127.3 (17.4)	79.4 (10.8)	25.8 (4.6)	33.9	16
EPIC- Norfolk - GWAS	2,100	54	57.2 (7.8)	136.7 (19.1)	83.9 (11.9)	26.3 (3.9)	45.6	16
Fenland	1,401	56	45.0 (7.3)	122.8 (16.3)	75.5 (10.7)	27.1 (4.9)	18.8	5.5
InCHIANTI	562	55	56.9 (14.5)	138.4 (20.1)	81.4 (10.1)	27.1 (4.2)	59.6	23.7
KORA	1,644	51	52.5 (10.1)	133.4 (18.5)	81.8 (10.9)	27.3 (4.1)	20.9	17
NFBC1966*	4,761	52	31*	125.2 (13.8)	77.5 (11.7)	24.6 (4.2)	21.7	2
SardinIA	3,998	57	40.8 (15.3)	128.7 (28.4)	79.7 (17.3)	25.1 (4.6)	29.5	10
SHIP	3,310	53	45.0 (13.9)	133.1 (20.2)	83.5 (11.3)	26.9 (4.7)	40.9	16.3
SUVIMAX	1,823	60	50.5 (6.2)	120.9 (12.3)	78.0 (8.1)	23.5 (3.3)	19.0	0
TwinsUK	873	100	45.8 (11.9)	122.9 (15.4)	78.2 (10.3)	24.8 (4.6)	27.3	22

Study	N	% women	Age (SD) years	SBP (SD) mm Hg	DBP (SD) mm Hg	BMI (SD) kg/m <sup>2</sup>	% HTN <sup>#</sup>	% anti-hypertensive therapy
<b>Controls from case-control studies</b>								
DGI controls	1,277	51	56.1 (8.7)	133.3 (18.4)	80.1 (10.0)	26.7 (3.8)	41.4	18
FUSION NGT controls	1,038	49	58.2 (10.7)	139.4 (19.3)	81.5 (10.3)	27.1 (4.0)	51.8	21
MIGen controls	1,121	38	48.9 (8.3)	127.1 (17.8)	80.2 (11.6)	27.1 (5.2)	36.4	13.4
PROCARDIS controls	795	37	58.9 (6.9)	134.7 (18.6)	82.8 (10.0)	25.9 (3.70)	15.0	2
<b>Stage 2-follow up</b>								
<b>2a. Cohorts with direct genotyping data</b>								
ARYA	736	52	57.0 (6.0)	125.0 (13.0)	72.0 (8.0)	25.0 (4.0)	15.8	1
BRIGHT-HTN	2,445	59	57.1 (10.8)	153.9 (20.8)	94.0 (11.0)	27.4 (3.8)	100	91.2
BRIGHT-NT	673	77	55.5 (8.5)	111.1 (6.9)	71.2 (6.6)	24.4 (3.2)	0	0
EPIC-Italy	3,909	37	49.0 (7.6)	132.5 (15.5)	83.7 (9.0)	26.0 (3.6)	43.1	12.7
EPIC-Norfolk-REP	15,858	48	56.2 (7.6)	133.8 (17.5)	82.3 (11.0)	26.3 (3.8)	44	15
Finnrisk97	7,023	51	47.1 (12.4)	134.9 (19.4)	82.3 (11.3)	26.6 (4.5)	45.5	12.4
FUSION2	1,162	37	57.5 (6.8)	138.2 (19.5)	83.9 (10.1)	26.8 (3.8)	8.9	1
Lolipop (Europeans)	6,006	35	51.2 (10.3)	130.4 (19.1)	79.6 (10.6)	27.5 (5.1)	39.9	20
Lolipop (Indian Asians)	12,823	36	48.8 (9.9)	129.9 (19.1)	80.8 (10.8)	27.4 (4.5)	42.9	25
MDC-CC	5,330	58	57.4 (5.9)	141.0 (19.0)	87.0 (9.5)	25.7 (4.0)	63.8	17
METSIM	5,934	0	58.1 (6.0)	142.0 (17.9)	89.8 (10.2)	27.3 (4.2)	69.6	40.5
MPP <sup>**</sup>	14,249	34	45.3 (7.1)	125.0 (14.0)	83.0 (9.1)	24.4 (3.4)	34.8	4
PREVEND	7,272	51	47.5 (11.4)	127.7 (19.3)	73.6 (9.7)	25.9 (4.2)	22.0	13.7
Prospect-EPIC	1,680	100	57.0 (6.0)	133.0 (20.0)	79.0 (11.0)	26.0 (4.0)	42.4	NA
Utrecht Health Project	2,829	52	40.0 (12)	128.0 (19.0)	79.0 (11.0)	25.0 (4.0)	32.9	NA
<b>2b. Cohorts with <i>in silico</i> data</b>								
CHARGE <sup>***</sup>	29,136							

\* Subjects from the North Finland Birth Cohort 1966 were examined at age 31 and from the British 1958 Birth Cohort samples were examined at ages 44–45.

\*\* The Malmö Preventive Project sample excludes all individuals who contributed to the Malmö Diet and Cancer Cardiovascular Arm (MDC-CC)

\*\*\* Full characteristics of CHARGE constituent cohorts are presented in manuscript submitted by CHARGE.

# Global BPgen definition of hypertension is SBP 140mm Hg or DBP 90mm Hg or taking anti-hypertensive medication.

Loci associated with blood pressure

Table 2

Shown is the top SNP for each independent locus associated with systolic or diastolic blood pressure ( $P < 5 \times 10^{-7}$ ) on joint analysis in up to 134,258 individuals of European ancestry from Global BPgen GWAS (stage 1), follow up genotyping (stage 2a) and *in silico* exchange with the CHARGE consortium (stage 2b). The eight genome-wide significant loci ( $P < 5 \times 10^{-8}$ ) are shown in **bold**. For stage 1 and 2b results based on imputed genotypes, an effective sample size is estimated to be the sum of the cohort-specific products of the imputation quality metric and the sample size. The total sample size is the sum of the effective sample sizes and the direct genotyping sample size. Effect sizes are on the mm Hg scale for increasing copy of the coded (alphabetically higher) allele as estimated by the beta coefficient in linear regression. The proportion of variance explained by each SNP is shown ( $r^2$ ). Meta-analysis was performed using inverse variance weighting. Note that loci 10q21 and 15q24 show results for two SNPs selected for validation genotyping in an interim analysis (rs1530440, rs1378942) that were genome-wide significant on joint analysis of stage 1+2a+2b. These two SNPs are highly correlated with alternate SNPs at the locus (rs4590817, rs4886606, respectively) with slightly stronger significance in the final stage 1 meta-analysis. The originally selected SNPs are shown throughout the text for consistency.

Chromosome	Genes Nearby	BP Trait	SNP ID (pos NCBI35) function	Coded allele	Stage	Coded allele freq	N	Beta (SE) mm Hg	P	Beta (SE)	P	N total
1p36	MTHFR											
	CLCN6	SBP	rs17367504	G	1	0.14	34,158	-0.79 (0.17)	1×10 <sup>-5</sup>			
	NPPA											
	NPPB		(11,797,044)		2a	0.16	19,751	-0.93 (0.22)	2×10 <sup>-5</sup>		r <sup>2</sup> =0.07%	
	AGTRAP		intron MTHFR		2b	0.16	29,064	-0.85 (0.20)	3×10 <sup>-5</sup>	-0.85 (0.11)	2×10 <sup>-13</sup>	82,973
10q24	c10orf26	SBP	rs11191548	T	1	0.91	33,123	1.17 (0.23)	3×10 <sup>-7</sup>			
	CYP17A1											
	c10orf32											
	AS3MT		(104,836,168)		2a	0.91	71,225	1.19 (0.15)	9×10 <sup>-15</sup>		r <sup>2</sup> =0.08%	
	CNNM2											
17q21	NT5C2		intergenic CNNM2/NT5C2		2b	0.92	28,204	1.05 (0.27)	9×10 <sup>-5</sup>	1.16 (0.12)	7×10 <sup>-24</sup>	132,552
		SBP	rs12946454	T	1	0.28	32,120	0.68 (0.15)	4×10 <sup>-6</sup>			
	PLCD3											
	ACBD4											
	HEXIM1		(40,563,647)		2a	0.25	17,877	0.43 (0.21)	0.045		r <sup>2</sup> =0.04%	
	HEXIM2											

Chromosome	Genes Nearby	BP Trait	SNP ID (pos NCBI35) function	Coded allele	Stage	N	Beta (SE) mm Hg	P	Beta (SE)	P	N total
<i>FMNLI</i>											
3q26	<i>MDS1</i>	DBP	intron <i>PLCD3</i>		2b	27,693	0.50 (0.17)	0.004	0.57 (0.10)	1×10 <sup>-8</sup>	77,690
			rs1918974	T	1	32,674	-0.28 (0.09)	1×10 <sup>-3</sup>			
			(170,648,590)		2a	26,910	-0.18 (0.08)	0.04		r <sup>2</sup> =0.03%	
			intron		2b	28,307	-0.35 (0.09)	8×10 <sup>-5</sup>	-0.27 (0.05)	8×10 <sup>-8</sup>	87,891
4q21	<i>PRDM8</i> <i>FGF5</i> <i>C4orf22</i>	DBP	rs16998073	T	1	26,106	0.65 (0.11)	7×10 <sup>-9</sup>			
			(81,541,520)		2a	53,508	0.50 (0.07)	6×10 <sup>-13</sup>		r <sup>2</sup> =0.09%	
			upstream <i>FGF5</i>		2b	22,009	0.36 (0.12)	0.003	0.50 (0.05)	1×10 <sup>-21</sup>	101,623
10q21	<i>c10orf107</i> <i>TMEM26</i> <i>RTKN2</i> <i>RHOBTB1</i> <i>ARID5B</i>	DBP	rs1530440	T	1	32,718	-0.51 (0.11)	3×10 <sup>-6</sup>			
			(63,194,597)		2a	19,884	-0.21 (0.11)	0.05		r <sup>2</sup> =0.04%	
			intron <i>c10orf107</i>		2b	27,651	-0.44 (0.12)	1×10 <sup>-4</sup>	-0.39 (0.06)	1×10 <sup>-9</sup>	87,273
12q24	<i>SH2B3</i> <i>ATXN2</i>	DBP	rs653178	T	1	30,853	-0.46 (0.09)	1×10 <sup>-7</sup>			
			(110,470,476)		2a	19,689	-0.40 (0.10)	3×10 <sup>-5</sup>		r <sup>2</sup> =0.09%	
			intron <i>ATXN2</i>		2b	29,119	-0.50 (0.09)	2×10 <sup>-8</sup>	-0.46 (0.05)	3×10 <sup>-18</sup>	79,661
15q24	<i>CYP11A1</i> <i>CYP11A2</i> <i>CSK</i> <i>LMAN1L</i> <i>CPLX3</i>	DBP	rs1378942	C	1	34,126	0.48 (0.09)	6×10 <sup>-8</sup>			
			(72,864,420)		2a	71,086	0.41 (0.06)	2×10 <sup>-12</sup>		r <sup>2</sup> =0.07%	



Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Chromosome	Genes Nearby	BP Trait	SNP ID (pos NCBI35) function	Coded allele	Stage	Coded allele freq	N	Beta (SE) mm Hg	P	Beta (SE)	P	N total
	<i>ARID3b</i>		intron CSK		2b	0.33	29,046	0.43 (0.09)	$3\times10^{-6}$	0.43 (0.04)	$1\times10^{-23}$	134,258
17q21												
		DBP	rs16948048	G	1	0.39	34,052	0.40 (0.09)	$5\times10^{-6}$			
	<i>ZNF652</i>		(44,795,465)		2a	0.37	19,752	0.23 (0.10)	0.02		$r^2=0.04\%$	
	<i>PHB</i>											
			upstream ZNF652		2b	0.37	28,637	0.29 (0.09)	0.002	0.31 (0.05)	$5\times10^{-9}$	82,441

**Table 3**  
**Relationship of SNPs at 8 genome-wide significant loci to both blood pressure traits**

For each of eight SNPs, the upper row shows association statistics for the blood pressure trait used for the analysis in which they were selected (SBP or DBP). The lower row (in bold) shows the equivalent association statistics for the alternate blood pressure trait. Results are shown for the 34,433 individuals in the stage 1 Global BPgen GWAS samples.

SNP ID	Chr	Position (NCBI35)	Coded allele	Noncoded allele	Coded allele frequency	N (effective)	Trait	Beta mm Hg	SE	P
rs17367504	1	11,797,044	G	A	0.14	34,158	SBP	-0.79	0.18	$1 \times 10^{-5}$
							<b>DBP</b>	<b>-0.50</b>	<b>0.12</b>	<b><math>3 \times 10^{-5}</math></b>
rs11191548	10	104,836,168	T	C	0.91	33,123	SBP	1.17	0.22	$3 \times 10^{-7}$
							<b>DBP</b>	<b>0.56</b>	<b>0.15</b>	<b><math>2 \times 10^{-4}</math></b>
rs12946454	17	40,563,647	T	A	0.28	32,120	SBP	0.68	0.15	$4 \times 10^{-6}$
							<b>DBP</b>	<b>0.34</b>	<b>0.09</b>	<b><math>6 \times 10^{-4}</math></b>
rs16998073	4	81,541,520	T	A	0.21	26,106	DBP	0.65	0.11	$7 \times 10^{-9}$
							<b>SBP</b>	<b>0.74</b>	<b>0.17</b>	<b><math>1 \times 10^{-5}</math></b>
rs1530440	10	63,194,597	T	C	0.19	32,718	DBP	-0.51	0.11	$3 \times 10^{-6}$
							<b>SBP</b>	<b>-0.43</b>	<b>0.16</b>	<b><math>7 \times 10^{-3}</math></b>
rs653178	12	110,470,476	T	C	0.53	30,853	DBP	-0.46	0.09	$1 \times 10^{-7}$
							<b>SBP</b>	<b>-0.47</b>	<b>0.13</b>	<b><math>3 \times 10^{-4}</math></b>
rs1378942	15	72,864,420	C	A	0.36	34,126	DBP	0.48	0.09	$6 \times 10^{-8}$
							<b>SBP</b>	<b>0.62</b>	<b>0.13</b>	<b><math>2 \times 10^{-6}</math></b>
rs16948048	17	44,795,465	G	A	0.39	34,052	DBP	0.40	0.09	$5 \times 10^{-6}$
							<b>SBP</b>	<b>0.41</b>	<b>0.13</b>	<b><math>2 \times 10^{-3}</math></b>

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 4  
Association of 8 SBP- and DBP-associated loci with hypertension

Shown are the results for the top SNP from each genome-wide significant SBP or DBP locus from a logistic regression analysis of the odds of hypertension compared to normotension (see **Methods**). For comparison, the effect of the coded allele on the continuous blood pressure trait is shown. The inverse-variance-weighted meta-analysis results are shown. BP = blood pressure, OR = odds ratio.

SNP ID	Chr	position (NCBI35)	Continuous Trait	Coded allele	Coded allele frequency	Continuous BP effect	HTN OR	HTN 95% CI	HTN P	N
rs17367504	1	11,797,044	SBP	G	0.14	↓	0.89	0.86 – 0.93	2×10 <sup>-9</sup>	62,803
rs11191548	10	104,836,168	SBP	T	0.91	↑	1.16	1.11 – 1.21	3×10 <sup>-13</sup>	99,153
rs12946454	17	40,563,647	SBP	T	0.28	↑	1.07	1.04 – 1.11	2×10 <sup>-5</sup>	57,410
rs16998073	4	81,541,520	DBP	T	0.19	↑	1.10	1.07 – 1.13	7×10 <sup>-10</sup>	73,756
rs1530440	10	63,194,597	DBP	T	0.19	↓	0.95	0.91 – 0.98	2×10 <sup>-3</sup>	83,156
rs653178	12	110,470,476	DBP	T	0.53	↓	0.93	0.91 – 0.96	8×10 <sup>-7</sup>	60,030
rs1378942	15	72,864,420	DBP	C	0.37	↑	1.10	1.07 – 1.12	2×10 <sup>-14</sup>	99,802
rs16948048	17	44,795,465	DBP	G	0.39	↑	1.06	1.03 – 1.09	1×10 <sup>-4</sup>	62,411